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Extraction of Paeoniflorin from *Radix Paeoniae Alba* by Supercritical CO_2

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In this study the supercritical CO_2 extracting technology was applied to extract paeoniflorin from *Radix Paeoniae Alba*, single factor experiment was used to study the influence of ethanol concentration, ratio of modifier dosage, extracting pressure, extracting temperature, extracting time and flow rate of CO_2 on the extraction yield of paeoniflorin. The optimum extracting parameters were established with ethanol concentration of 95 %, ratio of modifier dosage of 2.5, extracting pressure of 25 MPa, extracting temperature of 50 °C, flow rate of CO_2 of 10 kg h^{-1} and extracting time of 1.5 h. The optimum technology for supercritical CO_2 extraction of paeoniflorin from *Radix Paeoniae Alba* was high efficient, extraction yield 0.99 %.

Keywords: Paeoniflorin, *Radix Paeoniae Alba*, Supercritical CO_2 extraction.

INTRODUCTION

Radix Paeoniae Alba is one of the most widely used traditional Chinese medicine (TCM). It is the dried root of *Paeonia lactiflora* Pall., family Ranunculaceae. It can nourish and stabilize the blood, pacify the liver, alleviate pain, reduce and smooth irritability, edginess and mood swings[1]. *Radix Paeoniae Alba* has been frequently used as an important ingredient in many traditional prescriptions. Paeoniflorin is the major bioactive constituent existing in *Radix Paeoniae Alba*. It has broad pharmacological effects, such as analgesic, antidiuretic, antiinflammatory, anticonvulsant, vasodilatic, *etc.*[1,2].

The extraction of paeoniflorin from *Radix Paeoniae Alba* has been accomplished by several extraction methods in the past. These include heating reflux extraction[3], homogenate extraction[4,5] and ultrasonic-assisted extraction[6], with water, methanol, ethanol and some mixtures as solvents. However, the main disadvantage of the traditional extraction lies in the complicated working procedure which increases the cost; repeated distillations prolong the heating time and accelerate oxidation of the extract. These organic solvents used are problematic in the extraction of paeoniflorin because of their toxicity, volatility and flammability. To overcome the above-mentioned problems, environment friendly techniques become attractive following the development of the "Green Chemstry". Supercritical fluid extraction (SFE) technique is one of such technology as an alternative to the traditional extraction of natural products[7-10]. Supercritical carbon dioxide (SC-CO_2) extraction offers a number of clear advantages such as less consumption of hostile organic solvents, reduction of the extraction time, avoidance of degradation of active compounds and production of cleaner extracts[11].

In this study, the supercritical CO_2 extracting technology has been applied to extract paeoniflorin from *Radix Paeoniae Alba*, single factor experiment was used to study the influence of ethanol concentration, ratio of modifier dosage, extracting pressure, extracting temperature, extracting time and flow rate of CO_2 on the extraction yield of paeoniflorin.

EXPERIMENTAL

Supercritical carbon dioxide extraction device(model: HL-0.5/50MPaIIIA; Hangzhou Huali Pump Industry Co. Ltd., Hangzhou, China); UV spectrophotometer (model: 2450; Shimadzu, Japan); Electronic analytical balance(model: AB204-N; Ohaus, America).

Paeoniflorin was supplied by National Institute for the Control of Phanmaceutical and Biological Products (Beijing, China). *Radix Paeoniae Alba* was purchased from Hangzhou Chinese Medicine Factory (Hangzhou, China). Carbon dioxide (99.0 %) was supplied by Hangzhou Minxing industrial and trading Co. Ltd. (Hangzhou, China). Ethanol (analytical grade) was supplied by Hangzhou Chemical Reagent Co., Ltd. (Hangzhou, China).

Supercritical CO_2 extraction: *Radix Paeoniae Alba* powder (50 g) was placed in the extraction vessel. After an initial air purge, liquefied carbon dioxide and modifier (ethanol) were pumped into the extraction vessel by a high-pressure pump to a given pressure, the temperature inside the vessel was raised to and maintained at the desired level by a heating jacket encasing the vessel and the CO_2 flow rate was maintained at a given amount. Fractional separation was obtained setting the separator at 5 MPa and 40 °C.

After the extraction was completed, the extraction vessel was depressurized and the extract was collected.

Preparation of standard stock solution and study of calibration curves: The standard stock solution was prepared by dissolving paeoniflorin in 80 % ethanol to make final concentration of 200 μg mL^{-1}. Different aliquots were taken from stock solution and diluted with 80 % ethanol separately to prepare series of concentrations from 0-20 μg mL^{-1}. The λ_{max} was found by UV spectrum of paeoniflorin in ethanol, in the range of 200-400 nm and it was found to be 230 nm. Absorbance was measured at 230 nm against 80 % ethanol as blank. The calibration curve was prepared by plotting absorbance *versus* concentration of paeoniflorin. The calibration curve was shown in Fig. 1.

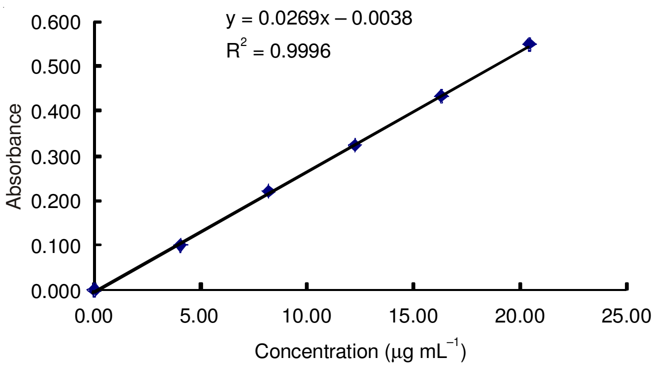

Fig. 1. Standard graph of paeoniflorin by UV spectrophotometric method

Preparation of sample solution: The product of supercritical CO_2 extraction was put into a 1000 mL clean dry volumetric flask and dissolved with 80 % ethanol. The obtained solution was used to determine the concentration of paeoniflorin by UV spectrophotometric method. Then the amount of paeoniflorin was calculated.

The yield is defined in eqn. (1). It was measured for each run and averaged for the three values.

$$\text{Yield} = \frac{\text{Paeoniflorin extracted in gram}}{\textit{Radix paeoniae alba}\text{ powder in gram}} \quad (1)$$

RESULTS AND DISCUSSION

Influence of ethanol concentration: Ethanol was used as an extracting modifier. The influence of ethanol concentration on the yield of paeoniflorin was given in Fig. 2 under other identical extaction conditions. The ethanol concentration was varied from 55 to 100 % while keeping ratio of modifier dosage at 2, extracting pressure at 20 MPa, extracting temperature at 40 °C, extracting time at 1.5 h and flow rate of CO_2 at 10 kg h^{-1}. The yield of paeoniflorin increases with ethanol concentration, reaching a maximum at 95 % and almost no changing thereafter.

Influence of ratio of modifier dosage: The ratio of ethanol to *Radix Paeoniae Alba* powder was varied from 1.5 to 3 keeping ethanol concentration at 95 %, extracting pressure at 20 MPa,

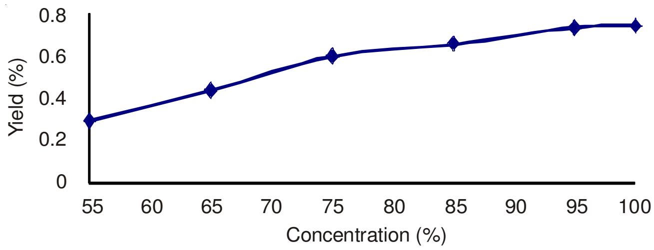

Fig. 2. Effects of ethanol concentration on yield of paeoniflorin

extracting time at 1.5 h and flow rate of CO_2 at 10 kg h^{-1}. The results are represented in Fig. 3. The yield of paeoniflorin reaches a maximum at 2.5.

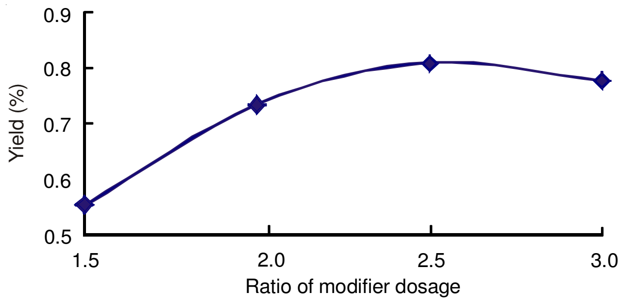

Fig. 3. Effects of ratio of modifier dosage on yield of paeoniflorin

Influence of extracting pressure: Pressure is a critical experimental variable for supercritical CO_2 extraction as it affects the density of the supercritical fluid, which is directly correlated to solute's solubility. The higher extraction pressure, at constant temperature, leads to a higher fluid density, increasing the solubility of the compounds[11]. In this assay, the influence of extracting pressure on the yield of paeoniflorin was given in Fig. 4 under other identical extaction conditions. The extracting pressure was varied from 15 to 30 MPa while keeping ethanol concentration at 95 %, ratio of modifier dosage at 2.5, extracting temperature at 40 °C, extracting time at 1.5 h and flow rate of CO_2 at 10 kg h^{-1}. The yield of paeoniflorin increases with extracting pressure, reaching a maximum at 25 MPa. With further increase in pressure, little decreased yield was observed.

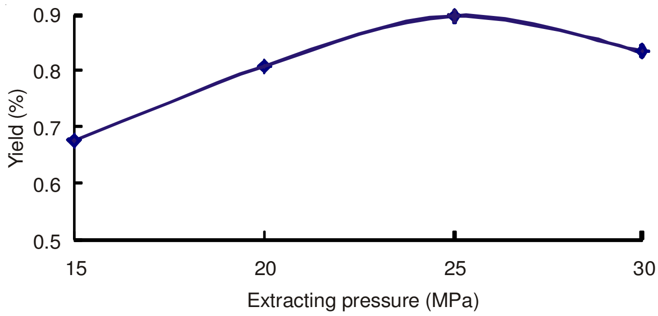

Fig. 4. Effects of extracting pressure on yield of paeoniflorin

Influence of extracting temperature: The effect of the extracting temperature is demonstrated in Fig. 5. The extracting temperature was varied from 40 to 55 °C keeping ethanol concentration at 95 %, ratio of modifier dosage at 2.5, extracting pressure at 25 MPa, extracting time at 1.5 h and

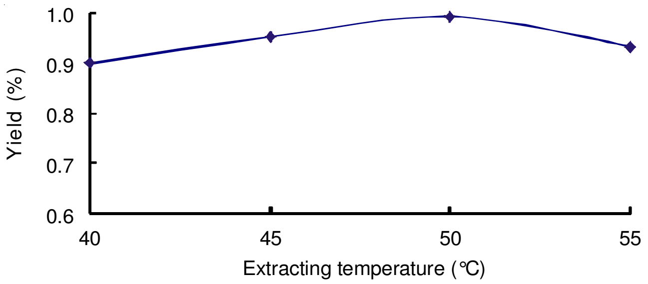

Fig. 5. Effects of extracting temperature on yield of paeoniflorin

flow rate of CO_2 at 10 kg h^{-1}. With the increase in temperature from 40 to 50 °C, the yield of paeoniflorin increases from 0.90 to 0.99 %. With further increase in temperature, little decreased yield of paeoniflorin was observed.

Influence of flow rate of CO_2: Flow rate of CO_2 was varied from 6 to 12 kg h^{-1} keeping ethanol concentration at 95 %, ratio of modifier dosage at 2.5, extracting pressure at 25 MPa, extracting time at 1.5 h and extracting temperature at 50 °C. The result was shown in Fig. 6. The yield of paeoniflorin increased from 0.60 to 0.99 % with the change in flow rate of CO_2 from 6 to 10 kg h^{-1}. With further increase in flow rate of CO_2, a slightly decrease in yield was observed.

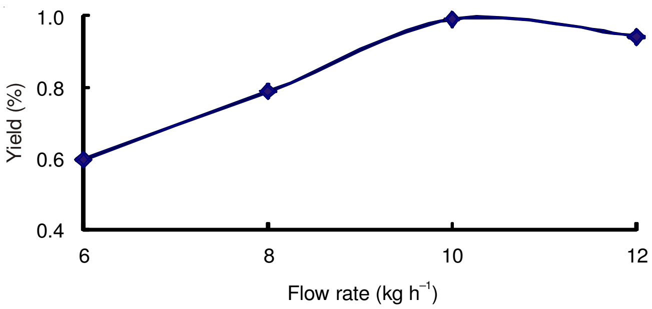

Fig. 6. Effects of flow rate on yield of paeoniflorin

Influence of extracting time: The influence of extracting time on yield of paeoniflorin was given in Fig. 7 under other identical extracting conditions. A gradual rise in yield was seen with increase in duration of extracting time. As seen from Fig. 7, in 1.5 h of extracting time, 0.99 % of yield is obtained, where as at the end of 2 h only 1 % of the extraction is complete. This suggests that 1.5 h is sufficient to optimize the extracting parameters.

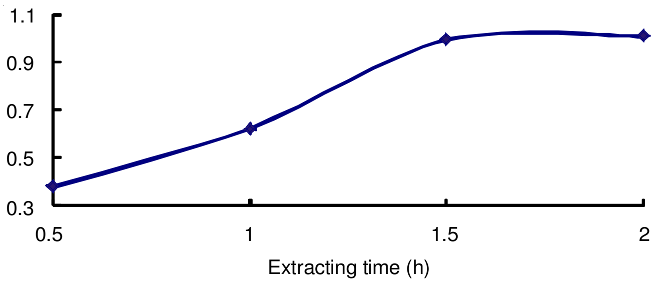

Fig. 7. Effects of extracting time on yield of paeoniflorin

Conclusion

The optimum extracting parameters were established with ethanol concentration of 95 %, ratio of modifier dosage of 2.5, extracting pressure of 25 MPa, extracting temperature of 50 °C, flow rate of CO_2 of 10 kg h^{-1} and extracting time of 1.5 h. The optimum technology for supercritical CO_2 extraction of paeoniflorin from *Radix Paeoniae Alba* was high efficient, extraction yield 0.99 %.

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